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Fat and Oil Microscopy

By VIRGIL C. MEHLENBACHER

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Microscopic studies of fats and oils have not occupied the thought and time of very many investigators. In view of our constant effort to improve and enlarge the scope of our methods for fat and oil analysis, coupled with the fact that in many instances our present procedures are insufficient to provide all of the information desired, it would seem that we can well afford to give some consideration to other means of adding to the applicability of these methods.

The advantages which should accrue from the development of reliable procedures for the microscopic analysis and identification of fatty substances are practically identical with those which in general apply to all microscopic methods, and these are not a few. To enumerate some of the more outstanding, it may be suggested that the time required for microscopic procedures is to the time required in the usual macro methods as minutes is to hours. Further than this, microscopic methods often permit a degree of accuracy, especially in the detection of slight changes in character, impurities, and adulterants present, which are well nigh impossible with the usual chemical methods. Very often the amount of sample available is so small as to prevent a complete and confirmatory analysis. This would worry the microscopist in but very few instances. Where a few ounces might be needed for a complete chemical analysis, a few drops and at the most a few milliliters would suffice for a microscopic examination. Last but not least, let us remember that the microscope, in any of its applications, offers the possibility of direct visual examination of whatever the substance. This is not permitted by but very few other known methods of analysis.

The literature reveals that the soap needles and crystallized soaps have been

studied with the microscope by various workers including Green,¹ Rosenthaler,² MacLennan,³ Hartwich and Uhlmann,⁴ and McClung.⁵

A method of long standing for the detection of beef fat in lard consists of the crystallization of the sample from the proper ethereal solvent followed by an examination of the crystals under the microscope.⁶

Hink's method for the qualitative detection of cocoanut oil has been described by Elsdon.⁷ The procedure is rather an elaborate process of crystallization to obtain the proper and identifying crystals. Trimen has reported on a further application of this same method.⁸

The American Association of Agricultural Chemists describe a microscopic procedure for the examination of butter fat.⁹

Lewkowitsch has described the microscopic appearance of some fats and oils and also discussed the procedure for examination of the unsaponifiable matter for the detection of cholesterol and phytosterol.¹⁰

Leach has also discussed means of examination of fats under the microscope.¹¹

Green has discussed fat and oil microscopy in general and has especially recommended phenylhydrazine as a reagent for producing characteristic crystals.¹² This latter phase of Green's work was based on previous studies made by Van Alphen which had to do with the reactions of phenylhydrazine with aldehydes and ketones occurring in fats.¹³ The writer's results with this reagent were not as satisfactory as it had been hoped, but this statement must not be taken as conclusive as there may be possibilities of further development along this line.

Staining methods have been used by those interested in biochemistry and physiology for the identification of the glycer-

ides as such for some time.¹⁴ Although these methods do have use in the qualitative detection of fats in the presence of non-fatty substances, their application to the selective differentiation of oils and fats is as yet unproved. Green has reported little success in this direction.¹⁵ Among the dyes which have been used in fat and oil work are the azo-ortho-phenols and azo-beta-naphthols such as Sudan III and Sudan IV.¹⁶ These substances are not acidic or basic and as such are not salt forming. However, the quinoid form does prove to be fat soluble and it is to this that the fat staining ability is due. Nile blue sulphate commonly called the Lorraine Smith Fat Stain is also used and the technique serves to distinguish between neutral fats and fatty acids.¹⁷ Osmic acid may be used to differentiate between the saturated and unsaturated fats, but this acid is very difficult, even hazardous, to handle.¹⁸ Stains may be very often used as a mounting medium when no other substance of the proper refractive index is available so as to increase the visibility of the specimen.

Perhaps the chief reason that the microscope has been so little used is because of the general crystalline habits and nature of the fats and oils. As a rule we do not consider that these crystallize in definite outline, and it is true that in a majority of cases perfect crystals do not result. However, Bragg by X-ray analysis has shown that when a long chain hydrocarbon is allowed to harden from a molten state the resulting mass is not one but many jumbled crystal flakes, and that, even in this jumble, there is a tendency of order.¹⁹ It is not necessary for us at this time to consider the order of growth or atomic arrangement, but the fundamental principle pertaining to the regularity of the outline produced by the growth is of vital significance to us in our



study. If there is a tendency to regularity in the building up of fat and oil crystals, and if we can control the formation of nuclei in such a way that these will be comparatively few in number so as to permit unhampered development of the crystal on all sides, a definite geometrical pattern should result. There is also good reason to believe that this geometric form will be influenced by the constitution and composition of the mother substance in such a way that definite crystals will result varying with the identity of the fatty substances. To prove whether or not this was true has been part of the object of this work.

In order to answer definitely the question of the applicability of fat and oil microscopy, a systematic survey and study of as many samples as possible tested by as many methods as possible is essential. Such a study must be made before the reliability of microscopic methods can be ascertained. As an indication of what such a survey must include the following is suggested:

1. The samples studied should include all, or as nearly all as possible, of the oils and fats which are apt to be encountered in industrial and commercial practice.

2. Many samples of each type of oil or fat should be selected to represent different sources, different methods of production, different seasons of production, and other possible variables.

3. The absolute and definite history of each sample must be known.

4. Each test or series of tests as well as each variable in procedure and technique must be performed with each sample.

5. All of the possible means of crystal formation should be studied, and all of the chemical, optical, and physical tests which are applicable to microscopy should be performed with each sample.

From an accumulation of such data as this it should be possible to arrive at a definite conclusion as to the general reliability and usefulness of microscopic methods and draw up final directions and methods for their application.

When almost any substance is submitted to microscopic study, there are in general two phases of the examination which may be considered. We can examine the geometrical form of the characteristic patterns or crystals which should result from crystallization by one means or another, and we may measure and define the optical and physical properties and constants of these crystals. These two general procedures may be further subdivided. Characteristic crystals, forms and patterns may be obtained in several ways including reactions with proper reagents such as to form definite crystals, by crystallization from proper media, and also by direct crystallization on the slide at the required temperatures. Included in the measurement of optical and physical

properties should be the observation and measurement of thermal phenomena by use of the cold or hot stage.²² Thermal phenomena consists chiefly of melting and solidifying points, transformation points as exhibited by substances which have more than one crystalline allotropic modification, and chemical reactions which are dependent upon temperature conditions. In this connection, we may well consider too the "liquid crystal" (anisotropic liquids) phenomena which is significant with long chain organic compounds, including soaps and waxes.²³ These are best studied with the polarizing microscope and a hot or cold stage. An examination between crossed nicols should be made as well as study and measurement of the optical properties.²⁴ The specimen may be examined with a polarizer after removing the analyzer.²⁵ The determination of refractive index²⁶ should be included as well as the effect of various solvents and the solution rates therein, and any other properties which are characteristic for specific crystals.

In June, 1936, the writer reported some of his results obtained following a study of several phases of fat and oil microscopy. Among other things in this work, a study was made of most of the previously recommended methods, and in the final report, tables and photographs of the results were included.²⁷

The samples which were investigated included those which probably are most frequently used in present day practice. Those were olive, kapok, soya bean, rice bran or kome, perilla, corn, palm, peanut, walnut, hempseed, mustard, sesame, cottonseed, teaseed, babassu, cocoanut, linseed, rapeseed, lard, beef-fat, sardine, and whale oils, and several hydrogenated fats. The reactions of all of these have been studied microscopically with aqueous potassium hydroxide, aqueous sodium hydroxide, potassium hydroxide in ethyl alcohol, potassium hydroxide in butyl alcohol, the same with sodium hydroxide, mixtures of potassium hydroxide and sodium hydroxide in ethyl alcohol, the same in butyl alcohol, bromine, iodine, and phenylhydrazine. Crystallization from all of the solvents herein discussed has been studied. The fatty acids have been prepared from these samples, and crystallized from solvents as well as directly on the slide. The reactions of the fatty acids with the reagents just discussed have been observed. Some observations of optical, physical, and thermal phenomena have been made, and crystallization on the slide of the original oils by use of a cold stage has also been resorted to. In several cases the unsaponifiable matter was separated and examined, after crystallization from alcohol, for any distinguishing characteristics.

It was found near the beginning of this problem that the dark field was especially applicable to the examination of fat and oil

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crystals and that it greatly improved the visibility and definition of the specimens. Dark field illumination differs from ordinary illumination in that the former causes the image to appear self-luminous. For a thorough discussion of the dark field the reader should refer to some such text as that of Gage² or Chamot and Mason.³ In all of our own work herein discussed, as well as the photographs included, dark field illumination was used.

The method of crystallizing from a suitable solvent is particularly applicable in the examination of fats, hydrogenated oils, and certain fatty acids. The general procedure is that of dissolving the sample in a suitable amount of the solvent, usually about five times the volume of the sample, closing the test tube with a cotton plug and allowing the sample to set at such a temperature as will permit a copious growth in about one hour. It is essential that a fairly rapid crystal formation take place so as to prevent fractional crystallization. What is needed is a formation of crystals representative of the true nature of the sample in question and not any one particular portion thereof, and this is best obtained by rapid growth due to supersaturation at the crystallizing temperature. Far more reliable and easily duplicable results have been found to be obtainable this way. It is, of course, possible to promote such a rapid crystal growth that the correct habit will be altered, and the characteristic outlines thus destroyed. This too must be avoided, and in such cases it is advisable to slow up the time somewhat, but it has never been necessary or advisable to extend this to more than two hours. If more than this is required at an ice box temperature, it has been found advisable to increase the concentration. Sometimes it has been found that removal of the precipitate by filtration, followed by resolution and reprecipitation on the slide has yielded more definite crystals, especially when dealing with some of the high melting point fatty acids.

Various solvents may be used including ethyl ether, alcohol, mixtures of these, absolute alcohol, petroleum ether, acetic acid, methyl or ethyl acetate and also occasionally amyl and butyl alcohol. When potassium hydroxide has been used as a reagent, alcohol has been found to be superior to water due to the more rapid evaporation rate. Butyl alcohol was found to be slightly superior to ethyl alcohol although very definite and practically identical crystals were obtained with either.

The crystals obtained by crystallization from any solvent may be removed from the test tube with an open end glass tube or a glass rod, but the writer prefers a platinum wire loop. In this way it is easier to remove the crystals with less adhering solvent, and the amount of precipitate taken from the

sample can also be better controlled. The crystals may be examined directly on the slide after covering with a cover glass, or they may be deposited in a small amount of clove oil, olive oil, or cottonseed oil on the slide. The writer prefers the use of clove oil.

Several reagents have been recommended by previous workers for the formation of characteristic crystals including sodium hydroxide, potassium hydroxide, mixtures of these, also mixtures of these with ammonium hydroxide, urethane, phenylhydrazine, and halogen mixtures. The writer has not found sodium hydroxide to be very satisfactory in the production of characteristic crystals, the trouble being that most of the crystals formed are very nearly identical. Potassium hydroxide has been found to be much better and in many cases quite capable of producing crystals varying with different oils and fats and reproductive for each.

The technique preferred by the writer when using a reagent such as potassium hydroxide in alcohol is first to place a drop of the liquid sample on an absolutely clean slide. Then about 3 to 5 millimeters from this place a drop of the reagent and tilt the slide in such a way to cause the reagent to flow toward the sample. When the outer edges have just touched, cover with a cover glass. At the point of junction of the two substances, the crystals will form. Usually these will develop within a few minutes if the temperature is about 20-25° C. Occasionally, especially with certain oils, a slight and gentle application of heat will promote crystal growth. In this case, however, extreme care must be observed or all of the distinguishing characteristics will be destroyed by a too rapid growth, thus preventing any possibility of identification.

Direct crystallization on the slide of various fatty acids has proven particularly productive in the formation of distinguishing crystals. The fatty acids may be prepared by the method of the American Oil Chemists' Society for the titer test except that special precautions must be observed in washing the acids after separation.⁴ If the experimenter is practiced in technique, it is possible and convenient to prepare the acids directly on the slide, working with a very small amount of sample.⁵ A great number of fatty acids, as separated from various oils or fats, have solidifying points such that they will crystallize on the slide without any external means of chilling. In such cases it is only necessary to apply the cover glass, and as soon as crystallization starts, which will be only a matter of minutes depending upon the temperature, examine under the microscope. When the fatty acids will not crystallize at room temperature, a cold storage should be resorted to, to induce solidification.

Figures 1 and 2 in the accompanying photographs are the fatty acids of lard and beef fat, respectively, prepared directly on the slide.

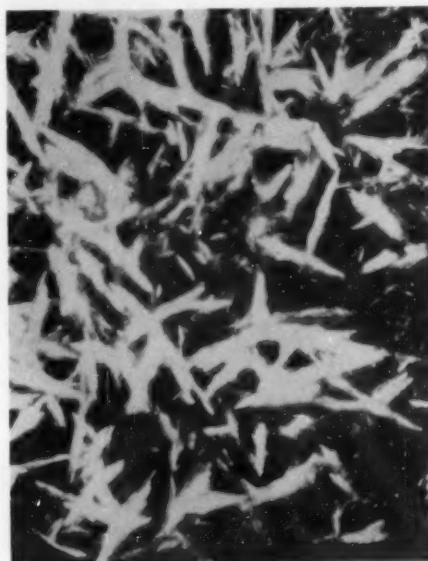


Fig. 1—Lard Fatty Acids.
80 X

At first glance it might be thought that these are very much alike. Whereas this may be true in part, close inspection will reveal a difference. It may be observed that the beef fat crystals more nearly resemble bundles of smaller crystals grouped together.

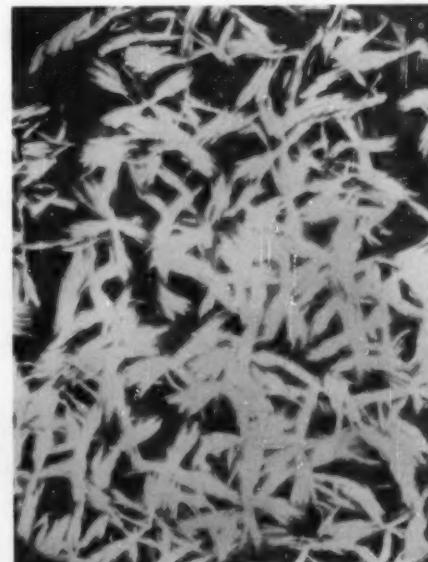


Fig. 2—Beef Fatty Acids.
80 X

This characteristic is usual in most beef fat fatty acid formations. The lard crystals, although there is somewhat the same tendency, are larger and appear to be individual and separate crystals of fat.

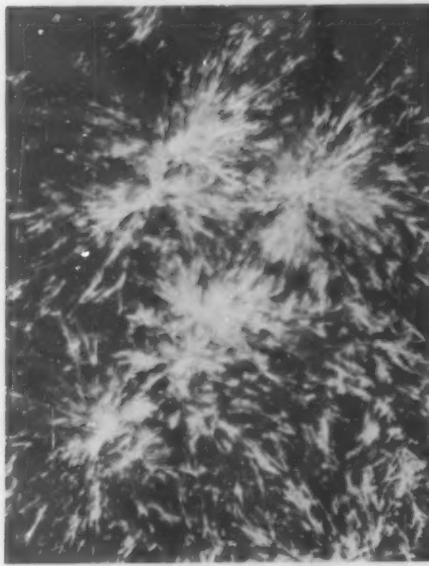


Fig. 3—Domestic Cottonseed Oil.
80 X

Figures 3-4-5-6 of cottonseed and kapok oil are the common spherocrystals of the fatty acids separated from these oils. It may be observed that there is no outstanding difference between oriental and domestic cottonseed oil but that both have a much heavier center growth than does kapok oil. Pure samples of either cottonseed or kapok oil cause no difficulty in identification.

Figure 7 is a photograph of the fatty acids of rice bran (kome) oil. This type of crystal formation is almost an oddity and has a tendency under proper conditions to grow to enormous size.

Figure 8 represents crystals of palm oil fatty acids which are masses or bunches of fine needles growing and pointing in every direction.

They are particularly characteristic for

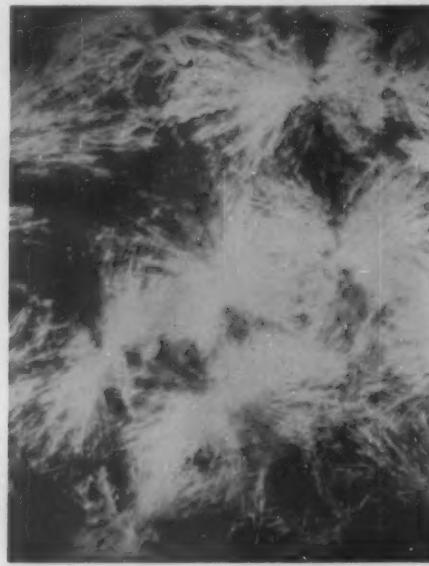


Fig. 4, No. 1—Oriental Cottonseed
Oil Fatty Acids.
80 X

this irregularity.

Babassu oil fatty acid crystals, Figure 9, are characteristic for the dumbbell shape and tufts at the extremities.

Olive oil fatty acid crystals, Figure 10, are again spherulitic but of an entirely different nature than those previously described.

Figures 11 and 12 are of sardine and whale oils fatty acids, respectively. The latter have a tendency to form concentric needles but with comparative few needles per crystal.

Figure 13 is cocoanut oil fatty acids which resemble groups or bunches of crystals. These after having been freshly crystallized have an appearance of curvature in outline something like a figure eight cut in

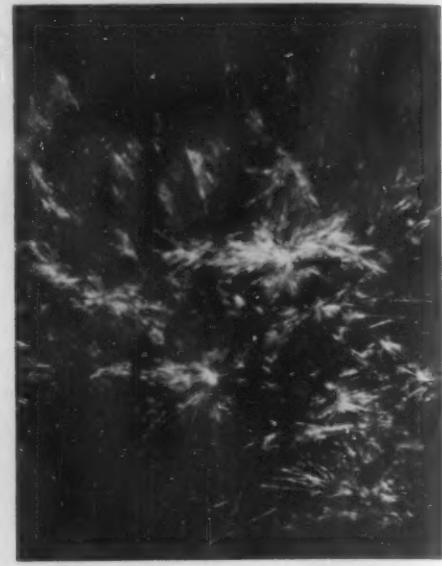


Fig. 5, No. 2—Oriental Cottonseed
Oil Fatty Acids.
80 X

two from top to bottom and turned back to back. This characteristic is lost on standing as is the case in the photograph shown.

Figures 14-15-16 are palm, cocoanut, and babassu oils, respectively, crystallized directly on the slide with a cold stage. The crystals of palm oil are small round balls of concentric needles which require high magnification to resolve and define. Cocoanut and babassu crystals are almost identical when formed in this manner.

Figures 17-18 are teaseed oil crystals formed with potassium hydroxide in butyl alcohol prepared as previously directed. These are some of the most easily formed as well as the most typical of all crystals obtained in this study.

(Continued on page 6)

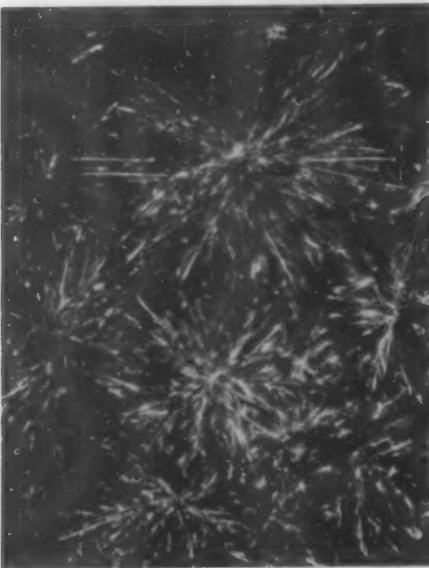


Fig. 6—Kapok Oil Fatty Acids.
80 X

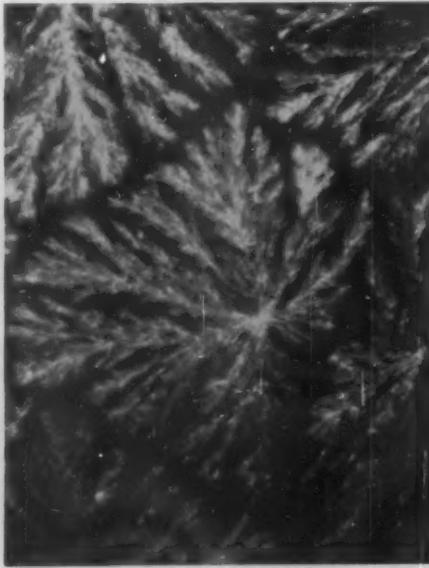


Fig. 7—Rice Bran Oil Fatty Acids.
80 X

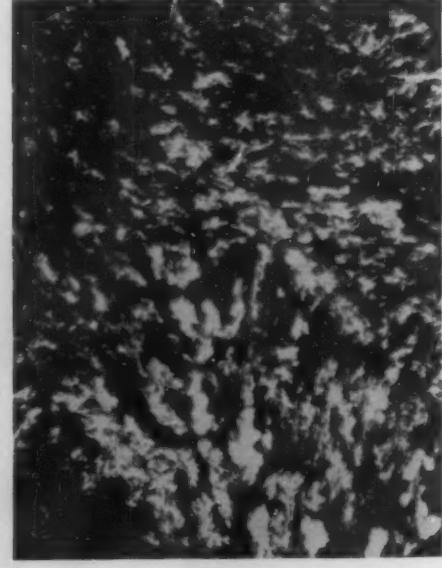


Fig. 8—Palm Oil Fatty Acids.
80 X

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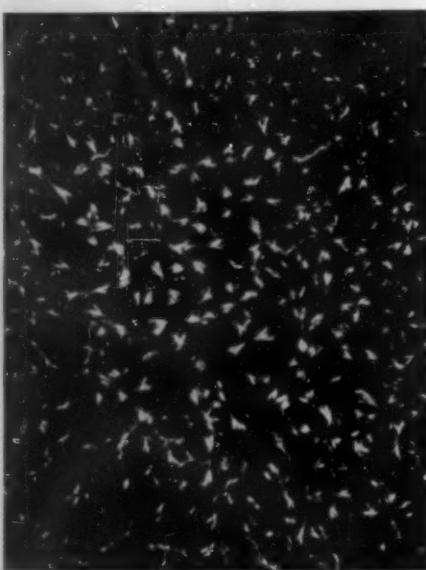


Fig. 9—Babassu Oil Fatty Acids.
 80 X

Car Manufacturers' Recommendations*

HUDSON TRANSMISSION AND OVERDRIVE LUBRICATION

On the 1941 Hudson models two separate filler plug holes are used, when overdrive is installed, one for the transmission and the other for the overdrive. A communicating passage in the form of a small hole is used between the two units to permit the passage of lubricant from one housing to the other. This communicating hole determines the oil level in the overdrive unit which should be about $\frac{1}{16}$ in. below the edge of the filler hole.

Whenever the transmission and overdrive are drained and refilled or they are checked for oil level, the following procedure should be carried out.

To Drain and Refill:

Be sure lubricant is warm so that it will flow more freely.

Remove both drain plugs and drain all old lubricant. Replace plugs.

Refill overdrive unit first, permitting level to come up to the bottom of the filler plug hole.

Next, refill transmission to level of bottom of the filler plug hole. Replace both plugs.

To Check Fluid Level:

Remove overdrive filler plug first and determine level, which should be about $\frac{1}{16}$ in. below edge of hole. This can be checked, if necessary, by using a $\frac{1}{4}$ in. flat steel gage with one end bent at right angles and insert into the case.

If level is low, refill to correct level.

Then check oil level in transmission and refill as required.

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Hudson has brought out a new type delivery car for door-to-door delivery of merchandise. All lubrication details, capacities, tire sizes, etc., are identical with standard passenger car chassis. There are a number of mechanical differences due to change in length, but these do not affect lubrication.

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Pontiac 1941 Models

At each lubrication period a small amount of Door-Ease or similar lubricant should be applied to the portion of door hinge post which contacts the plunger of the automatic dome lamp switch. This is necessary because the friction between these parts as the door is closed may cause the plunger of the switch to be bent sideways and ruin the switch unless a lubricating medium is used. Depress the plunger by hand; if it does not work freely, remove the switch by taking out the two retaining screws and put a few drops of oil inside the case and work the plunger so that the oil is distributed to contacting parts.

The hinges of the rear fender door (the gas tank filler cap cover) used on 1941 models need lubrication or the door may hold open slightly after the car has been used for 3,000 or 4,000 miles. If a few drops of oil are applied to the hinges now and then, while the door is being operated, the door will snap closed when released.

(Continued on page 7, col. 3)

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Fig. 10—Olive Oil Fatty Acids.
 80 X



Fig. 11—Sardine Oil Fatty Acids.
 80 X



Fig. 12—Whale Oil Fatty Acids.
80 X

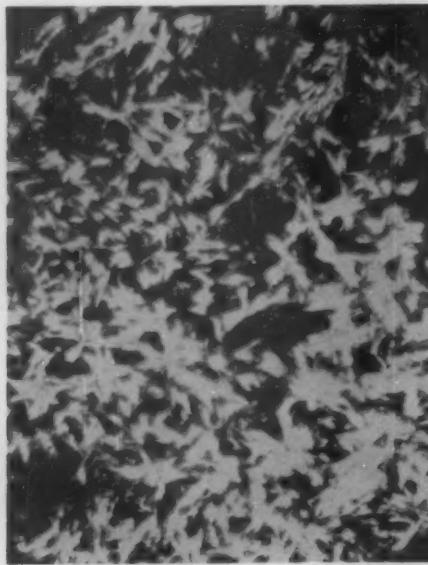


Fig. 13—Coconut Oil Fatty Acids.
80 X

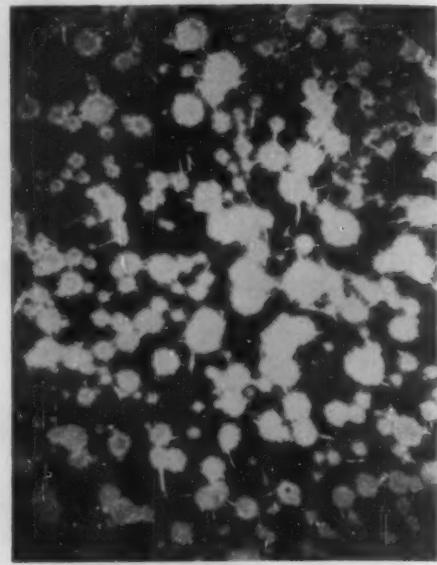


Fig. 14—Palm Oil Crystallized.
80 X

(Continued from page 4)

Figure 18 is a better picture of the individual crystals of teaseed—KOH.

Figures 19 and 20 represent corn and perilla oils, respectively, with potassium hydroxide in butyl alcohol.

We believe that the foregoing is good evidence of the fact that the microscope can be used to good advantage in the analysis and identification of fats and oils. It should be pointed out at this time that anyone attempting this work should compare the unknown samples with oils and fats of known purity. This at least, until such time as sufficient data of the optical and physical properties as well as photographs of known standard samples have been accumulated to provide for reference and comparison.

A few precautions might well be mentioned for the benefit of those who might be interested and attempt a furtherance of this work. A thorough knowledge of the principles of chemical microscopy, especially of the carefulness in technique, is a prerequisite. Experience with fat microscopy and especially in the differentiation of the results obtained is necessary and comes only with practice. When possible, it is advisable to examine both crude and refined oils of a given sample. A few oils change their characteristics during processing.

One of the most important questions to be answered will be the applicability of these methods to the analysis of mixtures. We have examined quite a number of known mixtures and have concluded that the possi-

bility to differentiate various fatty substances in mixture depends upon the composition and concentration of the same. Some are easily identified whereas others lose all known characteristics when mixed. The accumulation and tabulation of physical data should prove a great help in this case.

Although this is not the time at which a full report can be made of all the work which has been done, it is hoped that some of the advantages and possibilities may be seen by those interested. Much data yet remains to be gathered before definite conclusions can be drawn. However, there is definite evidence that a completion of this work should prove a helpful addition to our present methods and knowledge of fat and oil characteristics.

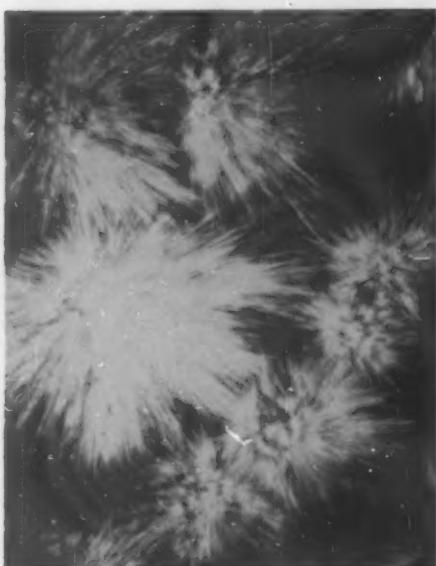


Fig. 15—Coconut Oil Crystallized.
80 X



Fig. 16—Babassu Oil Crystallized.
80 X

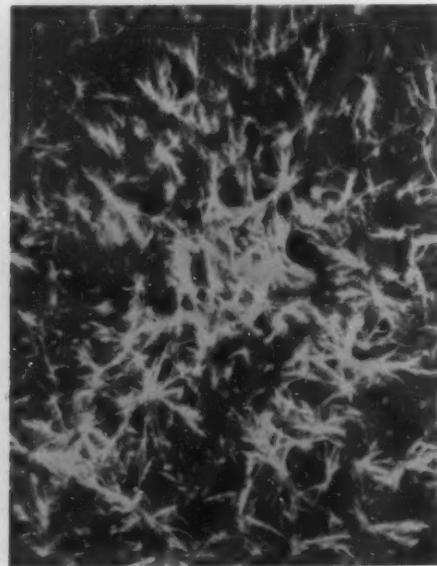


Fig. 17—Teaseed Oil with Potassium Hydroxide
80 X



Fig. 18—Teaseed Oil with Potassium Hydroxide.
80 X



Fig. 19—Corn Oil with Potassium Hydroxide.
80 X

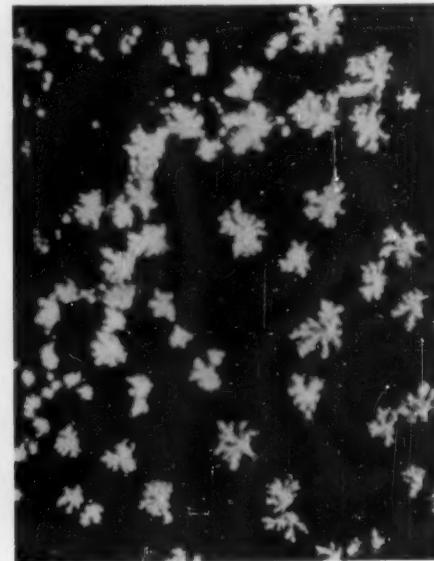


Fig. 20—Perilla Oil with Potassium Hydroxide.
80 X

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(Continued from page 5)
PONTIAC ANNOUNCES NEW
METROPOLITAN SEDAN

Pontiac recently introduced the "Metropolitan Sedan" — a new four-door sedan similar in appearance to the Custom Torpedo sedan, but on the DeLuxe Torpedo chassis. It is available with both six and eight cylinder engines.

The Metropolitan Torpedo sedan is an addition to the DeLuxe line. All other models, including the 4-door, 6-window sedan, are being continued.

Lubrication details, capacities of all units, tire sizes and pressures are the same for the Metropolitan Sedan as for other 1941 models.

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Accident Prevention Manual

The modern service station is no longer a simple gasoline and oil dispensary, but is a multi-phased business establishment catering to the many needs of the motorist.

Danger of accident lurks in the handling of inflammable fluids, in the operation of electrical equipment machinery, lifts, tools, etc. An accident may cause damage that could seriously cripple the station or put it out of business, either as a direct result of the accident or the payment of damages. An accident to an employee or customer curtails his income or terminates it forever.

Accidents can, to a great extent, be prevented.

All the lessons gained from accidents have been carefully studied by the Department of Accident Prevention of the American Petroleum Institute and worked up into a new edition of a 29 page book called the "API Accident-Prevention Manual of Service Stations (2nd Edition—1941)."

This manual classifies accidents, proposes means for preventing them and makes suggestions for best procedure if the accident does happen. It covers fully (with many illustrations) falls, sprains, burns, collisions, fires, gasoline delivery, smoking hazards, oil service, radiator service, pressure systems, anti-freeze, air service, battery service, lifts, lubrication service, building maintenance, tools, equipment, fire prevention, fire fighting and general safety.

Prepared in a convenient form for filing, pages 8 in. x 10½ in., price 25 cents. Write to the Department of Accident Prevention, American Petroleum Institute, 50 West 50th St., New York City.

ONE-THIRD OF ALL VEHICLES EVER MADE
ARE OPERATING TODAY

More than one-third of all the automobiles and trucks ever produced in the United States are still traveling the highways, the American Petroleum Industries Committee declares.

Since, 1900, the Committee asserts, almost 80,000,000 motor vehicles have been produced by the American automobile industry; prior to that date only a few thousand vehicles were manufactured. Thirty-seven per cent of these vehicles, 30,600,000, were registered and in operation last year, and about 23 per cent of these are estimated to be 10 years or more old.

Although more used motor vehicles are sold each year than new vehicles, the Committee finds that obsolete vehicles are being scrapped at a rate of two to two and one-half million vehicles annually.

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